

SUPPLEMENTARY MATERIAL

Unusual alkaloids of the highland species *Astragalus cryptanthus* Wedd. (Fabaceae)

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Two unusual caprolactam alkaloids, 3-(dimethylamino)hexahydro-2H-azepin-2-one and 3-(methylamino)-hexahydro-2H-azepin-2-one, were isolated from the aerial parts of *Astragalus cryptanthus* Wedd.; their structures were unambiguously determined based on data from extensive 1D and 2D NMR, GC-MS and FT-IR spectroscopic analyses. This is the first report of this alkaloid type in the genus *Astragalus*.

Keywords: Fabaceae; *Astragalus cryptanthus*; caprolactam alkaloids

Experimental

Plant material

Aerial parts of *A. cryptanthus* Wedd. were collected near Lake Chungará, Chile (18°15.104' S, 69°10.683' W, 4588 masl). Voucher specimens (LQE 10-20) were deposited in the herbarium at Laboratorio de Química Ecológica, Facultad de Ciencias, Universidad de Chile.

Extraction of alkaloids and analysis

Oven dried plant material (300 g) was extracted with 3 l of CH₃OH (analytical-reagent-grade, JTBaker, USA) by maceration in an ultrasonic bath (Power Sonic 405, Hwashin Technology, Korea) at medium intensity for 3 h at 30°C and subsequently with agitation overnight at 25°. The suspension obtained was filtered through a Büchner funnel using Whatman® grade 1 filter paper and the resulting extract was evaporated under reduced pressure in a rotary evaporator (41.4 g). The syrupy residue was agitated with 450 ml of 5%

HCl in an ultrasonic bath at medium intensity for 90 min at 30°C and at high intensity for 60 min at 30°, and filtered through paper Whatman® grade 1. For easier manipulation, the clear filtrate was divided in nine 50-ml fractions, each of which was washed with CHCl₃ (3x25 ml). Fractions (ca. 100 ml each) of the aqueous phase were adjusted to pH 10-11 with 25% NH₄OH and extracted with CHCl₃ (4x25 ml) until the organic extracts gave negative Dragendorff reaction. Finally, the grouped organic extracts were dried with anhydrous Na₂SO₄ and evaporation of the solvent yielded an extract potentially containing alkaloids (0.14 g, 0.05% relative to the dry plant material).

The alkaloidal extract was fractionated by silica gel column chromatography eluted with CHCl₃:CH₃OH:12% v/v NH₄OH (9:0.6:0.4), yielding fifty-six fractions labelled F-1 to F-56. Dragendorff reagent was used to assess for presence of alkaloids. Alkaloids were concentrated in fractions F-29 to F-44. They were grouped (9.8 mg) and seeded onto a 20 x 20 cm preparative glass chromatoplate coated with 1 mm silica gel (Merck, 60 F₂₅₄). The plates were developed twice using CHCl₃:CH₃OH:12% v/v NH₄OH (9:0.6:0.4), and examined under UV light at 254 and 365 nm. The two Dragendorff-positive bands were removed from the chromatoplates and extracted using chloroform. Subsequently, the suspensions were vacuum filtered and the filtrates dried with anhydrous Na₂SO₄. Finally, each extract was evaporated to dryness using a rotary evaporator, labeled and stored for subsequent analysis. Thus, 4.2 mg of alkaloid **1** (Rf= 0.35, 0.0014 %) and 4.7 mg of alkaloid **2** (Rf= 0.25, 0.0016%) were obtained. These two alkaloids were characterized through NMR, GC-MS and FT-IR analyses.

GC-MS analysis

GC/MS analysis was performed with a Shimadzu model GCMS-QP 2010 Ultra gas chromatograph (Shimadzu, Kyoto, Japon), equipped with an Rtx-5MS Crossbond 5% diphenyl 95% dimethyl polysiloxane (Restek, Bellefonte, PA, USA) capillary GC column (30 m length, 0.25 mm I.D., 0.25 µm film thickness). The GC was operated in the splitless injection mode using 2 µL injection volume. The column temperature was initially held at 30° C for 3 min, then raised at 25°C/min to 230° C, and maintained for 10 min at 230° C. The carrier gas was helium at a flow rate of 1.32 mL/min. The mass spectrometer used electron impact (EI) ionization mode (70 eV) with an emission current of 250 µA. The

temperatures of the injection port, ion source and transfer line were set at 250° C, 250° C and 280° C, respectively. Qualitative analysis of compounds was carried out comparing the retention indices and MS spectra for relevant peaks with the data in the NIST08 database.

NMR analysis

NMR spectra (both 1D and 2D) were obtained on a Bruker AVANCE 400 spectrometer (400 MHz for ¹H and 100 MHz for ¹³C) using TMS and the residual solvent peaks as internal standards.

FT-IR analysis

IR spectra were recorded as KBr discs on a FT-IR Raman Perkin–Elmer series 2000 instrument equipped with a deuterated triglycine sulfate detector (DTGS) and using a 4 cm⁻¹ spectral resolution.

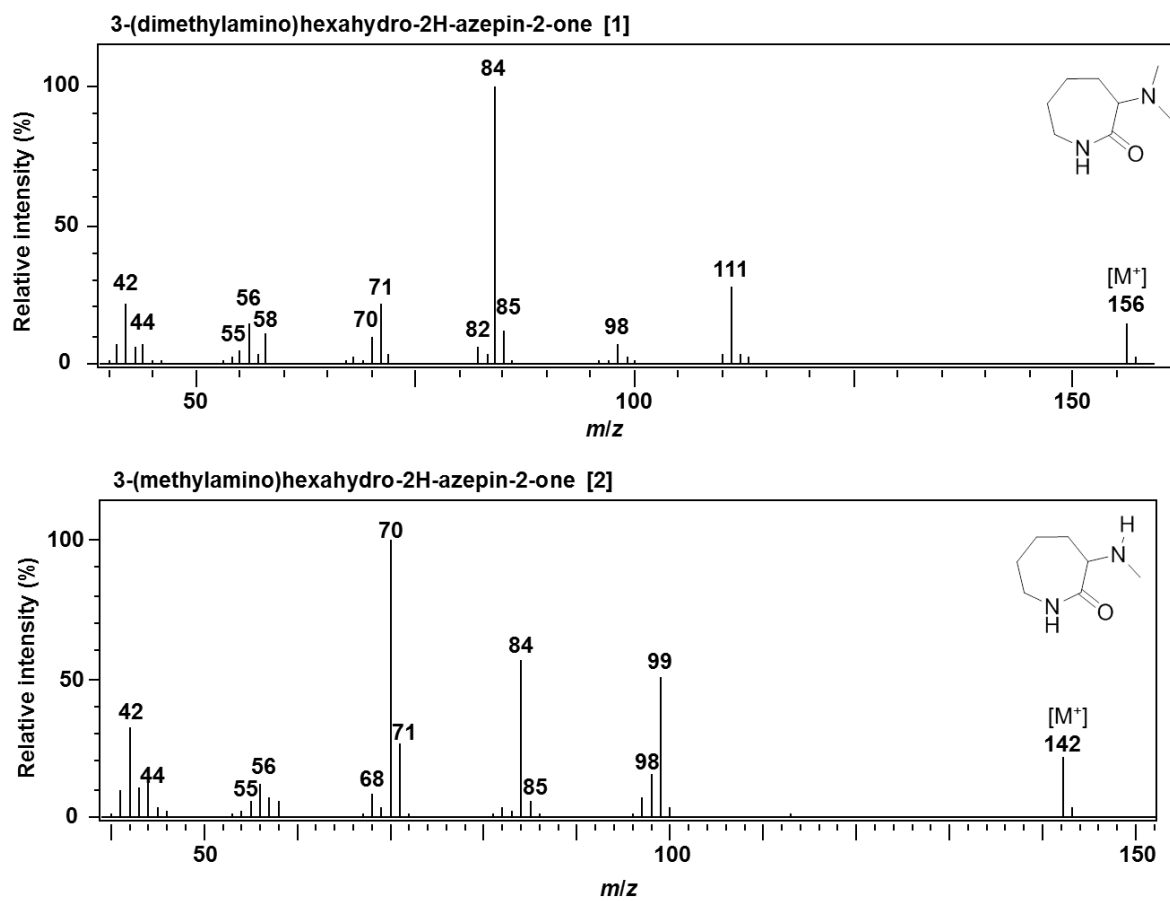


Figure S1. Mass spectra of alkaloids **1** and **2**.

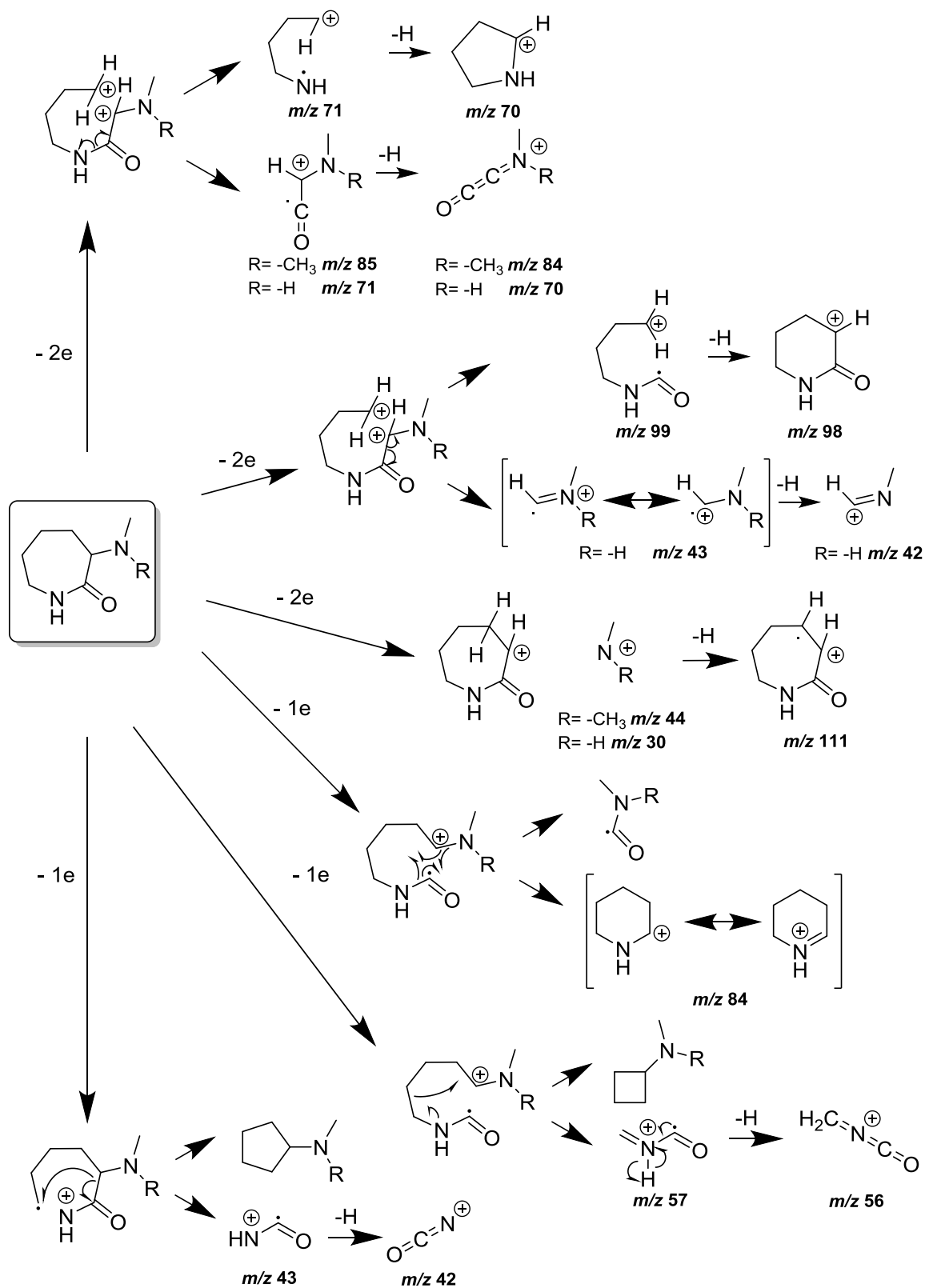


Figure S2. Proposed fragmentation patterns for alkaloids **1** and **2**.

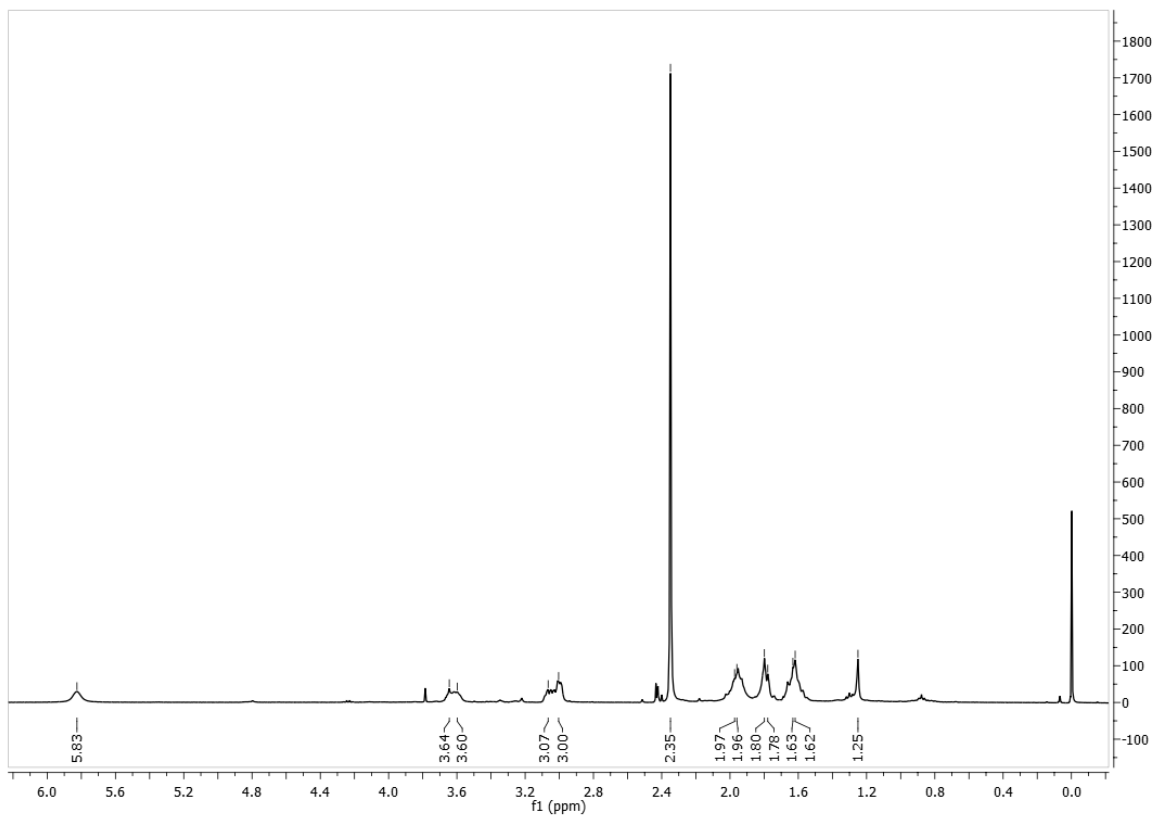


Figure S3-a. $^1\text{H-NMR}$ of 3-(dimethylamino)hexahydro-2H-azepin-2-one [1]

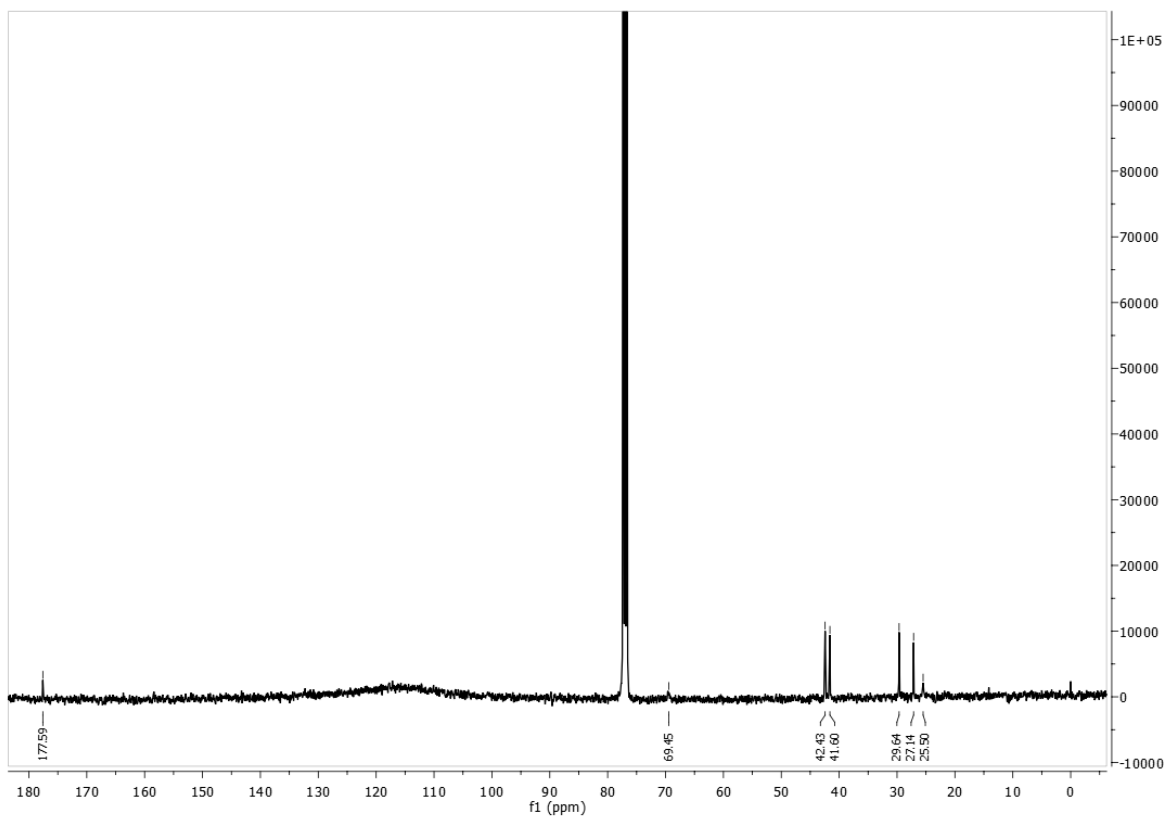


Figure S3-b. ^{13}C -NMR of 3-(dimethylamino)hexahydro-2H-azepin-2-one [1]

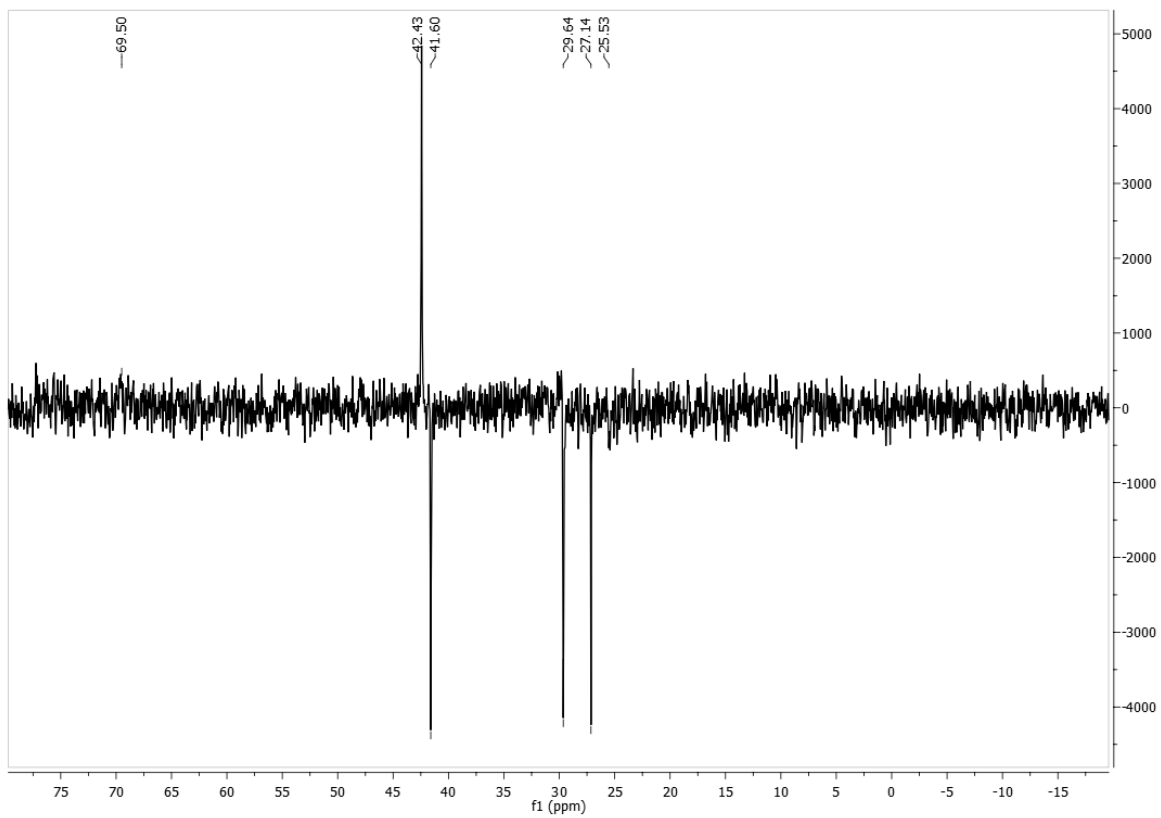


Figure S3-c. DEPT-135 of 3-(dimethylamino)hexahydro-2*H*-azepin-2-one [**1**]

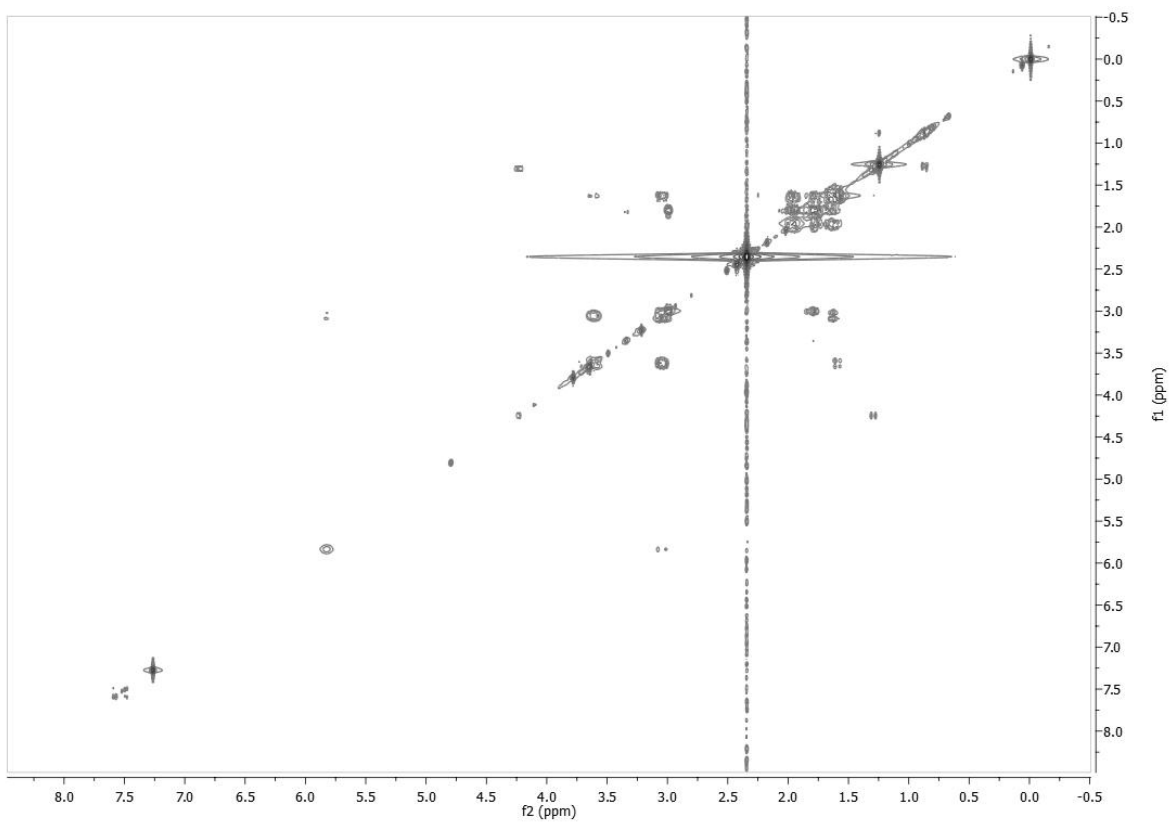


Figure S3-d. COSY of 3-(dimethylamino)hexahydro-2*H*-azepin-2-one [1]

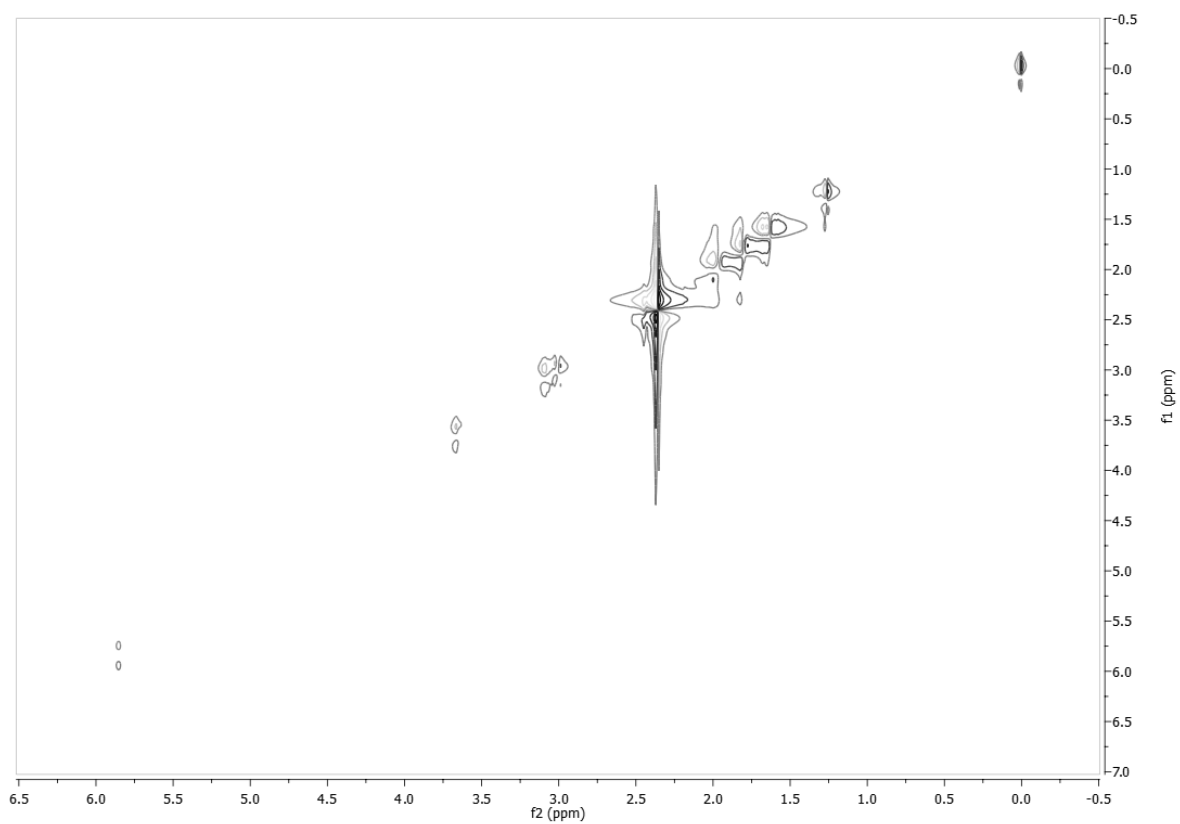


Figure S3-e. NOESY of 3-(dimethylamino)hexahydro-2*H*-azepin-2-one [1]

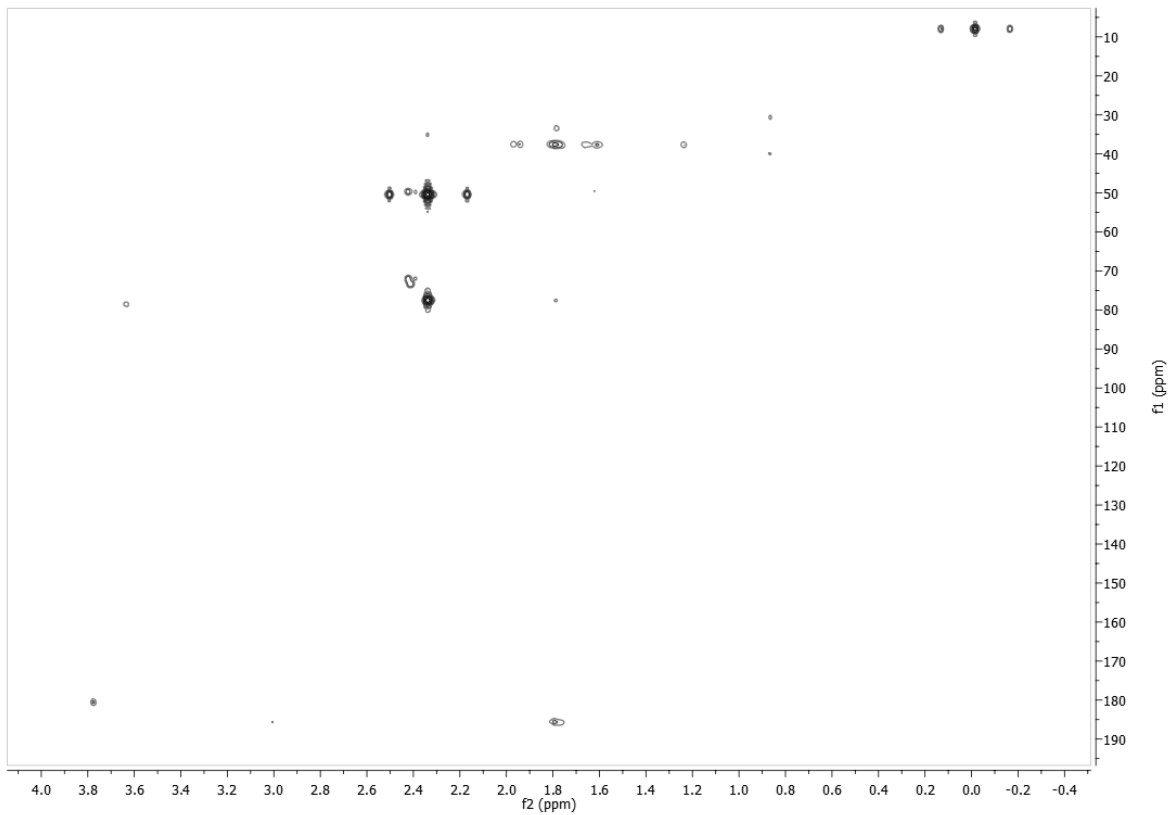


Figure S3-f. HMBC of 3-(dimethylamino)hexahydro-2*H*-azepin-2-one **[1]**

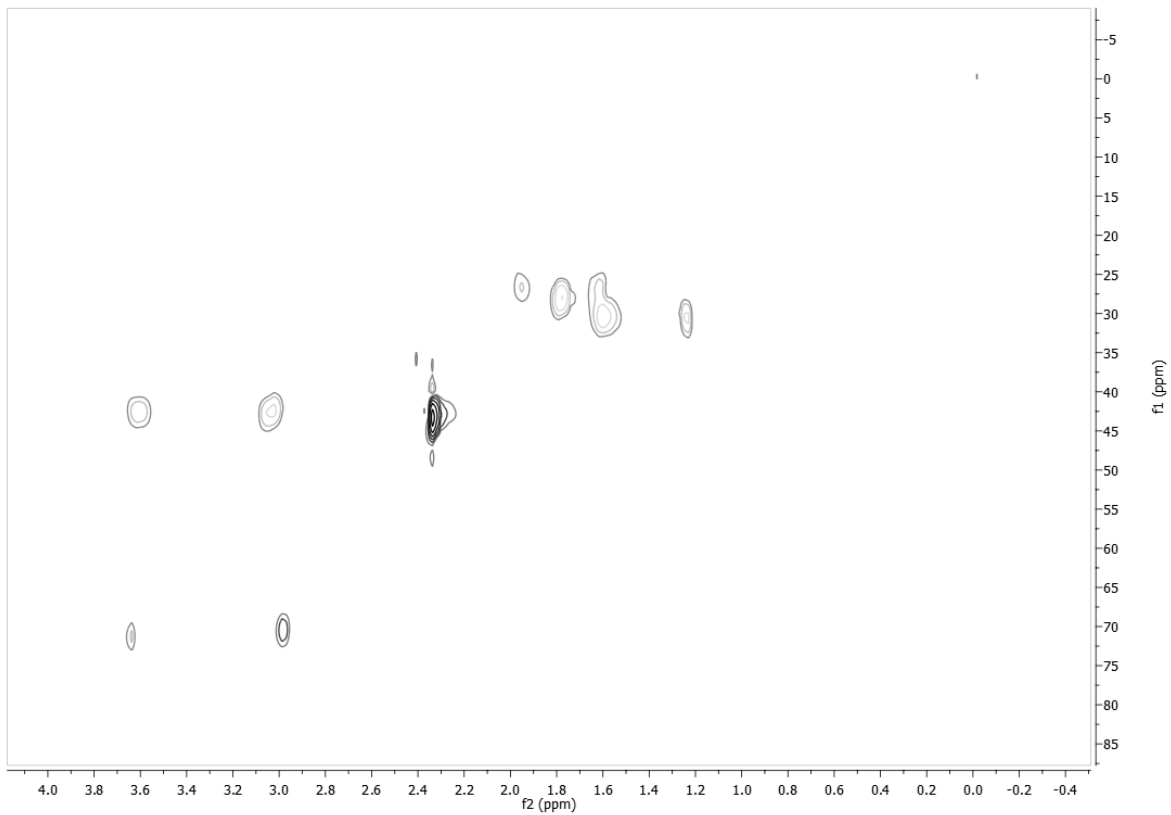


Figure S3-g. HSQC of 3-(dimethylamino)hexahydro-2*H*-azepin-2-one [1]

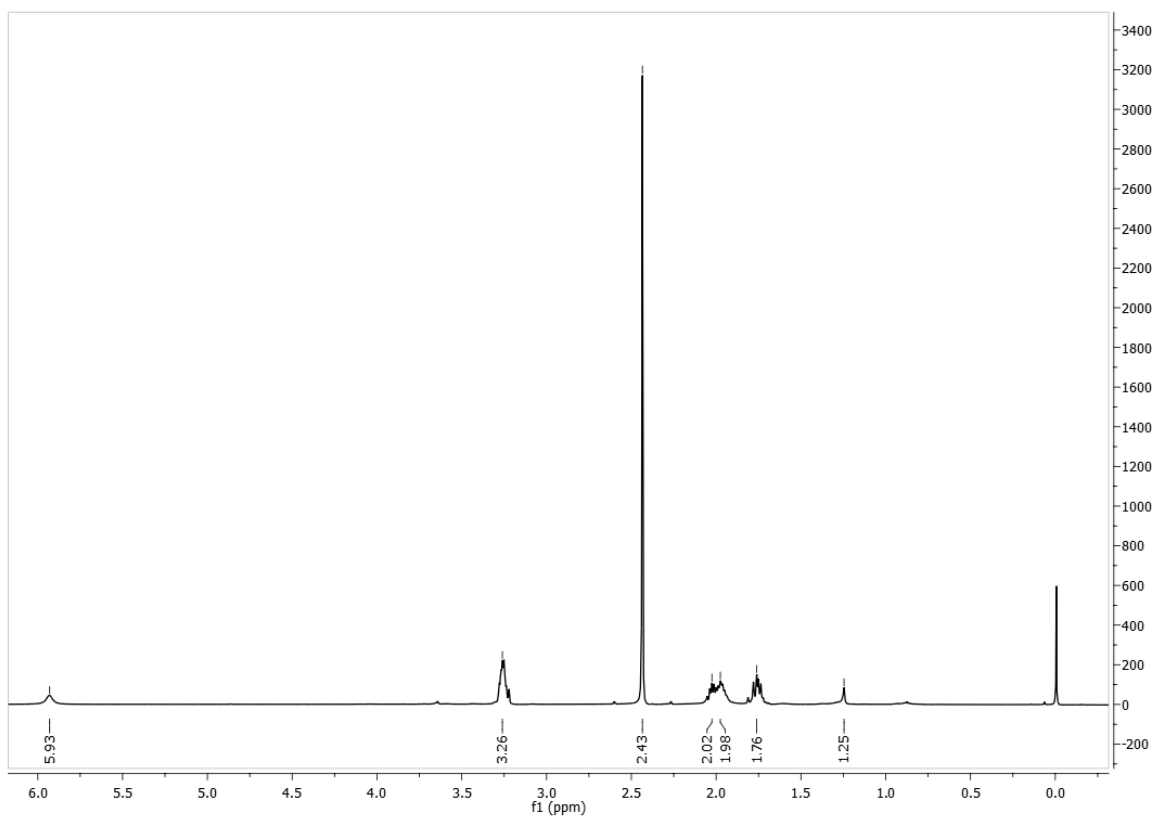


Figure S3-h. $^1\text{H-NMR}$ of 3-(methylamino)-hexahydro-2H-azepin-2-one [2]

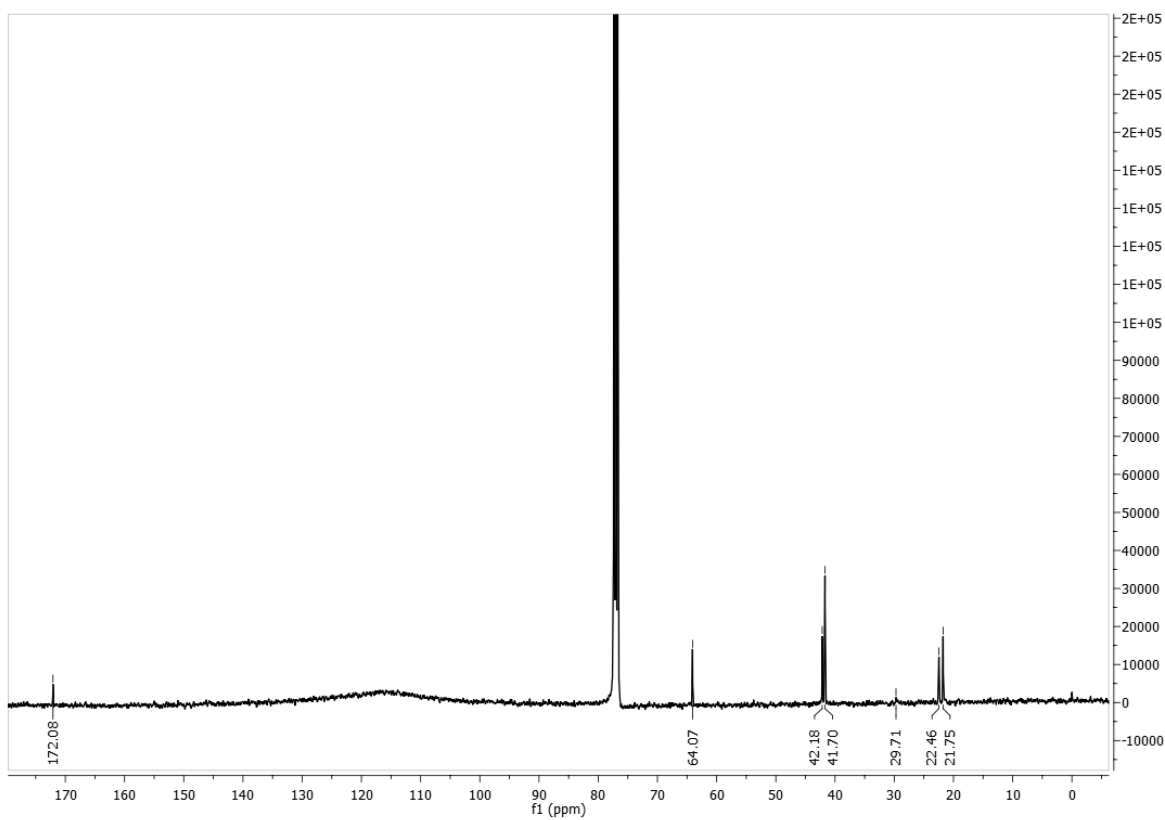


Figure S3-i. ^{13}C -NMR of 3-(methylamino)-hexahydro-2H-azepin-2-one [2]

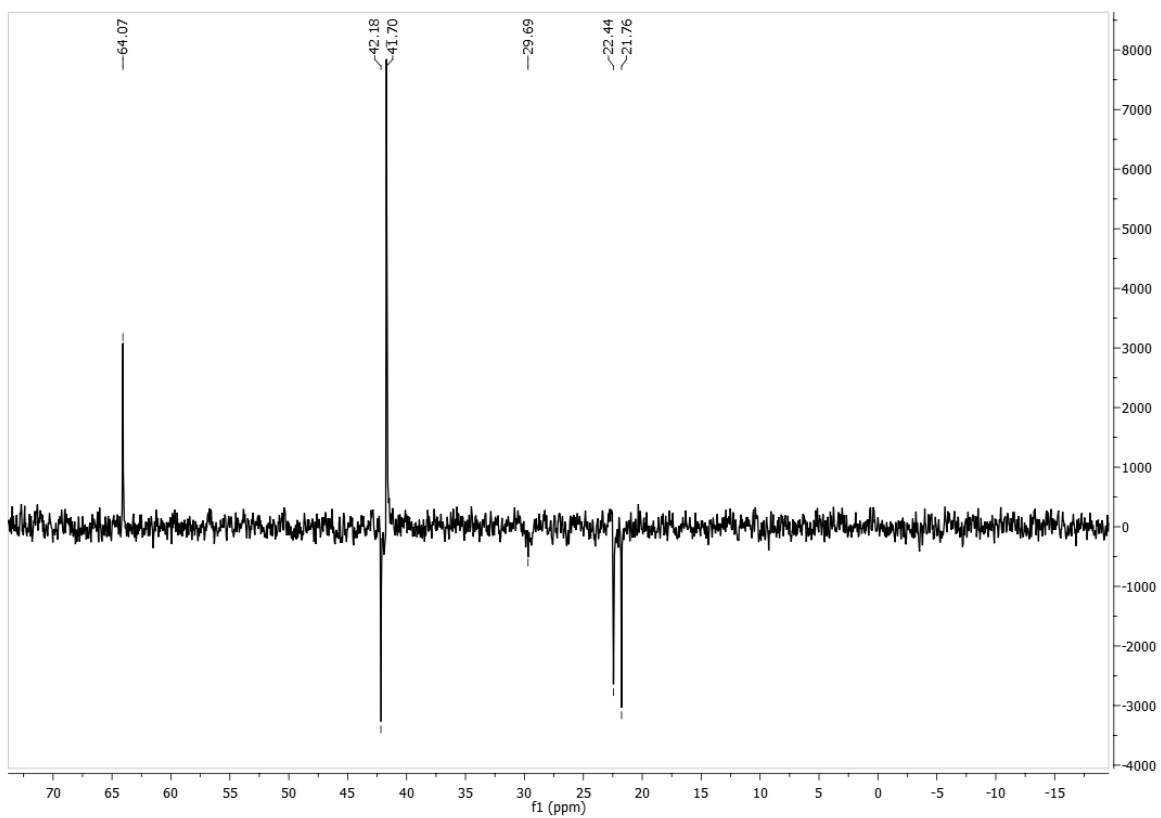


Figure S3-j. DEPT-135 of 3-(methylamino)-hexahydro-2*H*-azepin-2-one [2]

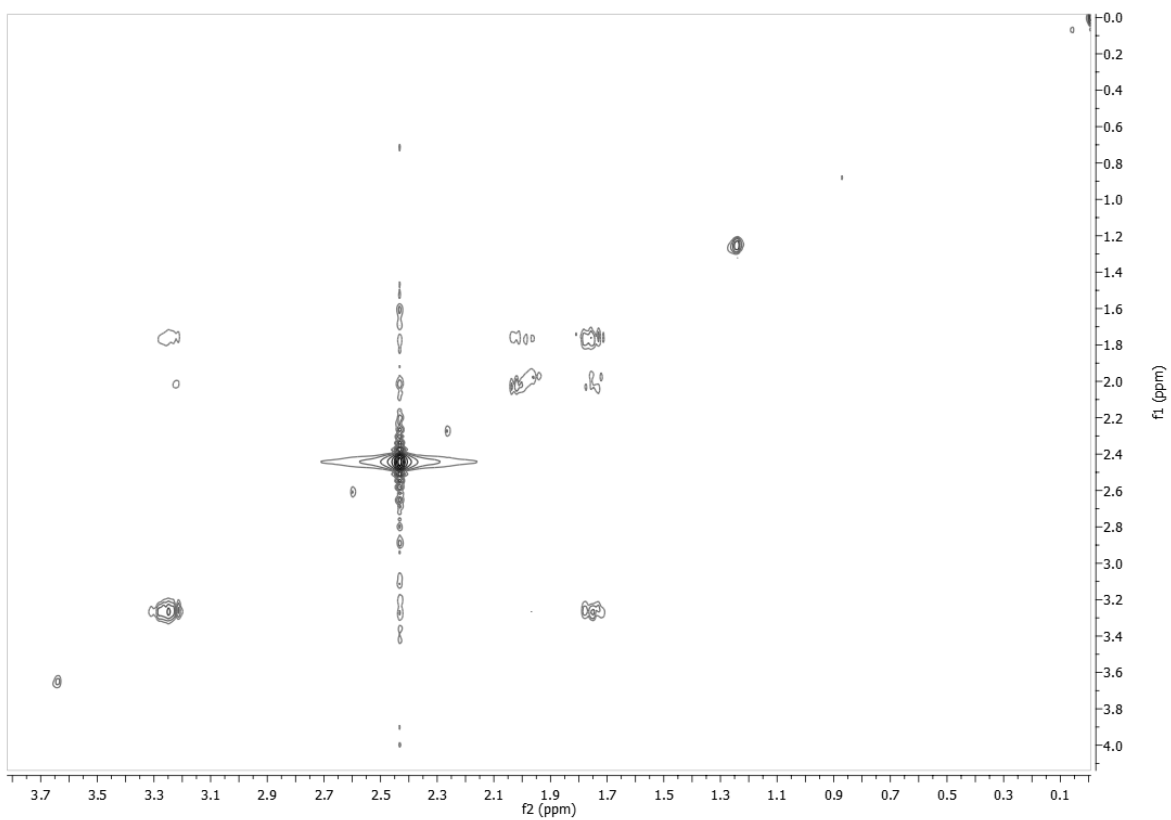


Figure S3-k. COSY of 3-(methylamino)-hexahydro-2*H*-azepin-2-one [2]

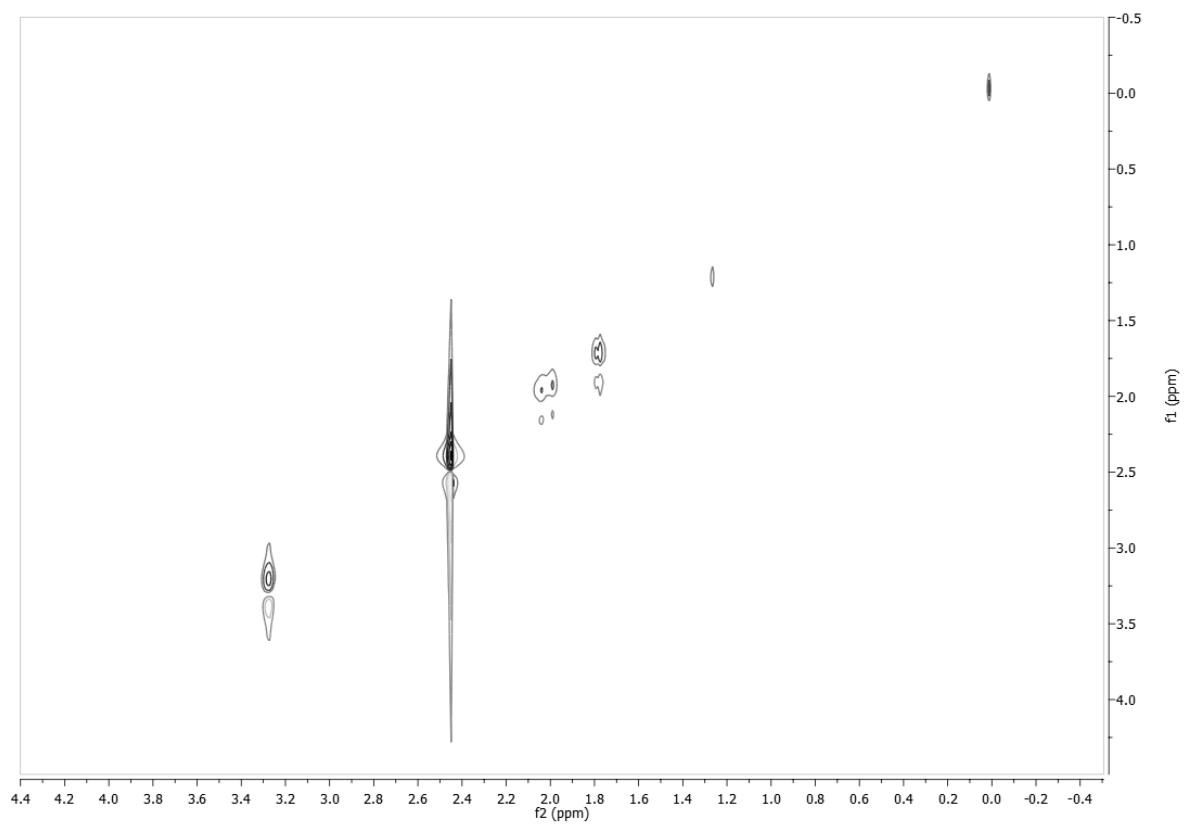


Figure S3-1. NOESY of 3-(methylamino)-hexahydro-2*H*-azepin-2-one [2]

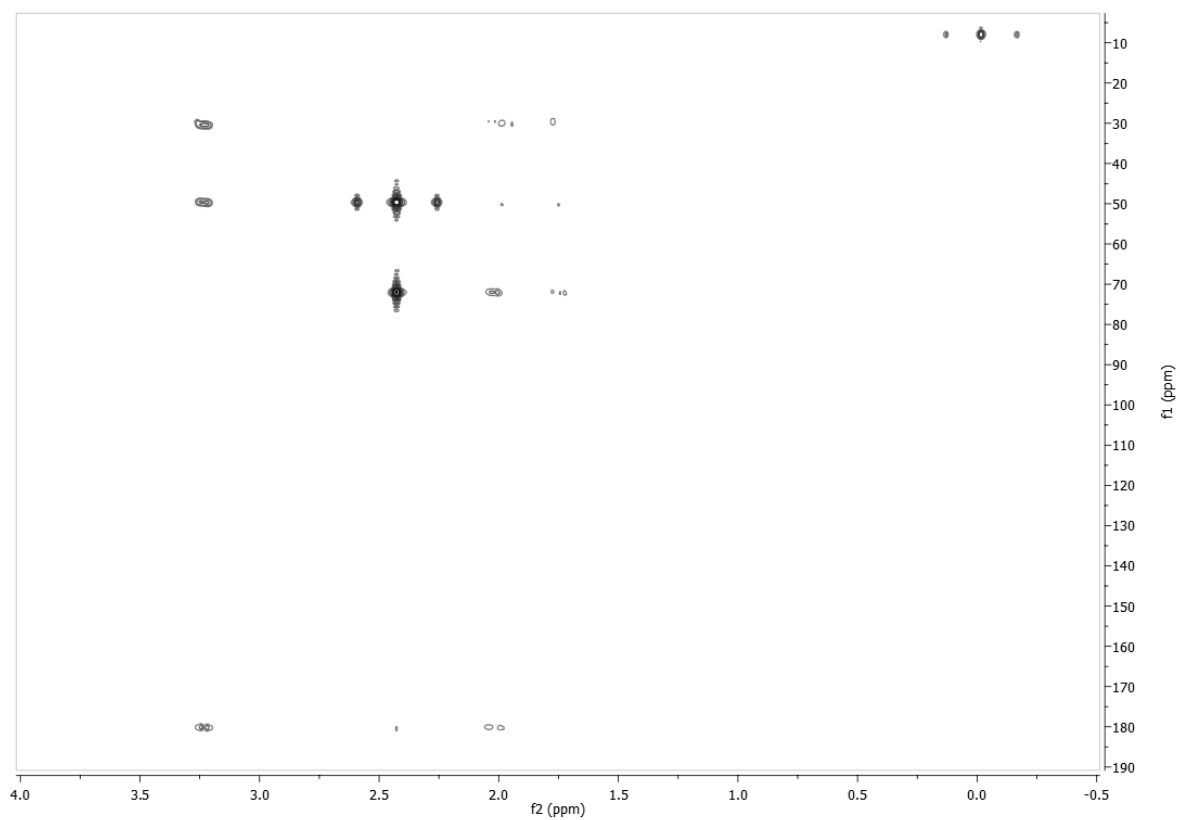


Figure S3-m. HMBC of 3-(methylamino)-hexahydro-2*H*-azepin-2-one [2]

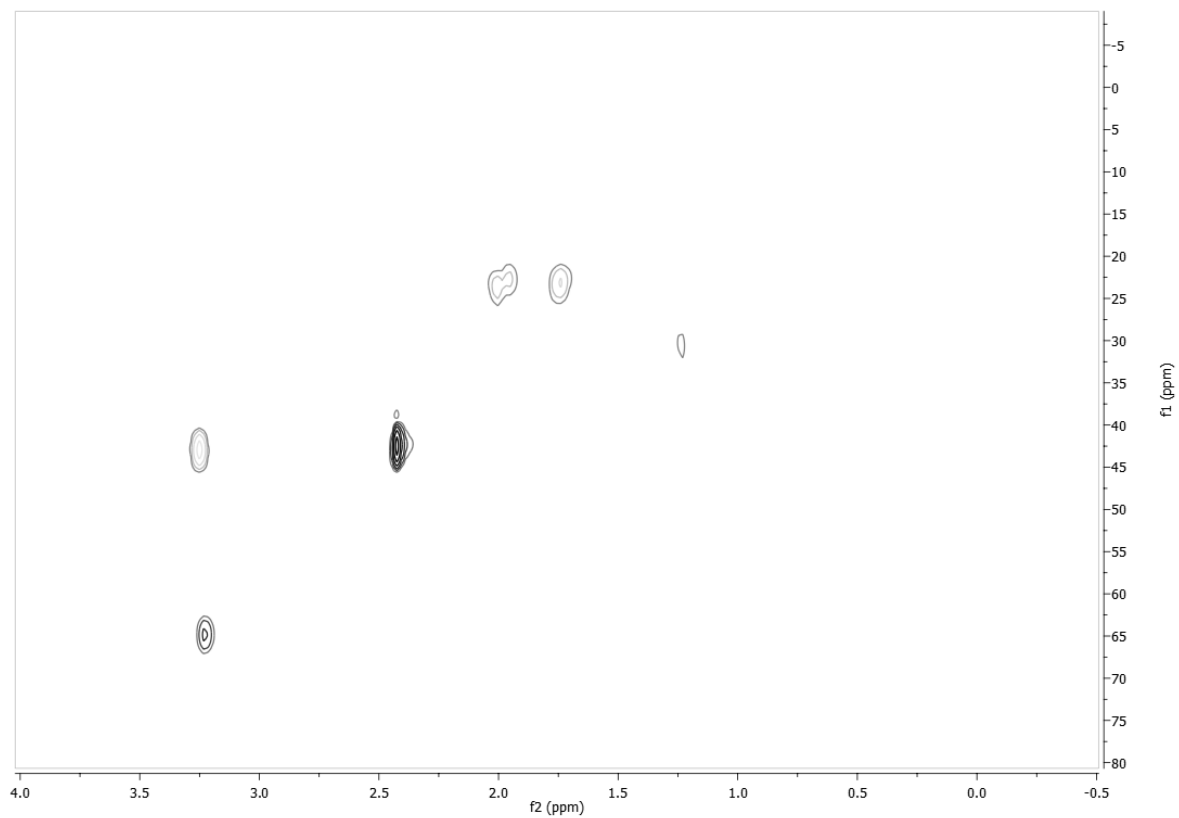


Figure S3-n. HSQC of 3-(methylamino)-hexahydro-2*H*-azepin-2-one [2]

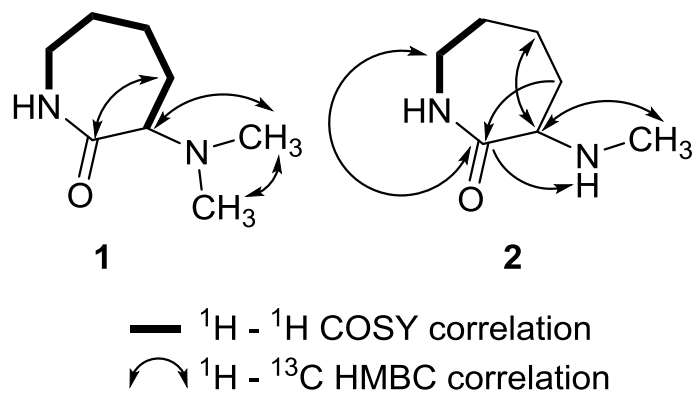


Figure S4. Key COSY and HMBC correlations for alkaloids **1** and **2**.